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Precopulatory guarding-time of the male amphipod *Eogammarus oclairi*: effect of population structure

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Abstract This study shows that population structure affects guarding time in the amphipod *Eogammarus oclairi* Bousfield. In the laboratory, mean guarding time depended on the male weight-frequency distribution, operational sex ratio, and male density. It was longer when the male population encompassed a broad range of weights than when the weight distribution was narrower; however, guarding time did not differ when the population's weight distribution was narrow and composed of either small or large males. Mean guarding time varied between a few hours when the operational sex ratio (male:female) was 1:3 to > 7 d when the sex ratio was 2:1. It was also affected by male density, increasing as male density increased. The results suggest that the stimulus to guard depends on the intensity of male–male competition.

Introduction

Amplexus occurs in many crustaceans (e.g. *Cancer* spp., Edwards 1966; Snow and Neilsen 1966; *Callinectes sapidus*, Gleeson 1979; *Carcinus maenas*, Cheung 1966; Eales 1974; *Homarus americanus*, Atema and Engstrom 1971; *Pandalus platyceros*, Hoffman 1973; and *Alpheus*

heterochaelis, Schein 1975). Hard-shelled males carry pre-molt females for up to several days. Mating occurs shortly after the female molts.

Several species of amphipods and isopods also exhibit amplexus, during which the swimming male carries the passive female for varying times (Borowsky 1984). Most female amphipods are receptive for only a short period after molting (Birkhead and Clarkson 1980; Borowsky 1988). Ovulation occurs while the female exoskeleton is still flexible enough to permit eggs to pass through the gonopores; since there is no sperm storage (Borowsky 1988), copulation must immediately precede ovulation. Accordingly, the guarding phase usually terminates with insemination. Fecundity is significantly reduced if fertilization is delayed even for a relatively short period (e.g. *Gammarus palustris*, Borowsky 1988; *Eogammarus oclairi*, Iribarne 1994).

Precopulatory guarding is costly for males in terms of reduced survival (Strong 1973; VanDolah 1978; Ridley and Thompson 1979; Wildish 1982), increased energy expenditure (Manning 1975; Calow 1979), reduced growth (Robinson and Doyle 1985), and time lost in search of other females (Parker 1974, 1978; Hunte et al. 1985). Thus the optimal strategy for a male should be to begin amplexus as close to the time of the female molt as possible.

The time of initiation of amplexus varies within species of amphipods (*Gammarus pulex*, Ward 1983; *G. lawrencianus*, Dunham et al. 1989) and isopods (*Assellus aquaticus* and *A. meridianus*, Manning 1980). Commencement of guarding in *G. lawrencianus* depends on both time since the male's last copulation (Dunham et al. 1989) and contact with female conspecifics during the postcopulatory phase (Dunham and Hursham 1991). Guarding time may also be influenced by other factors including the size structure of the population (e.g. *G. pulex*, Ward 1983), the operational sex ratio (OSR, e.g. *A. aquaticus* and *A. meridianus*, Manning 1980), searching time, cost of searching per unit of time, cost of guarding, number of eggs likely to be fertilized

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(usually a function of female size), and the possibility of displacement by another male.

The density of potential mates during the mating season also influences the mating system (Parker 1970; Wells 1977; Thornhill 1984; McLain and Boromisa 1987). Based on a computer simulation model, Crowley et al. (1991) concluded that increased availability of mates should promote assortative mating. Although evidence suggests that information on density may help to discriminate between alternative hypotheses concerning mating systems, density data are infrequently reported in mate-choice studies.

The pattern of mating in *Eogammarus oclairi* Bousfield is similar to that in other amplexing amphipods (Bousfield 1979). Laboratory experiments showed that *E. oclairi* males tend to select larger females, and larger males may either outcompete or displace smaller males in amplexus (Iribarne 1994). Interactions between a wandering male and an amplexing couple were influenced by how close the female was to molt, the size of the female, and the relative size of the intruding male compared to that of the paired male. Males increased their resistance to breaking off amplexus as females neared ecdysis. This suggests that assortative mating is mostly driven by male-male competition and that large male size is advantageous and under selective pressure (Iribarne 1994).

If interactions associated with mating in *Eogammarus oclairi* are competitive, the population structure (e.g. weight-frequency distribution, OSR, and density) should play an important role in shaping the mating system. In this study, we investigate the effect on guarding time in *E. oclairi* of the relative weight distribution of competing males, the local density, and the operational sex ratio.

Materials and methods

Eogammarus oclairi Bousfield were collected from the intertidal shell habitat of Grays Harbor's South Channel, Washington, USA (46° 56' N; 123° 55' W), between May and August 1992. Mating pairs were kept under laboratory conditions (8 h darkness:16 h daylight) until the females molted. They were kept in a 50-liter aerated aquarium filled with estuarine water (25 to 27‰ S, 14 to 16°C) that was changed every other day, and were fed green algae (*Enteromorpha* spp.) and fish food daily. Only those amphipods with a pairing history known for the previous two months were used in experiments. Since the female intermolt period lasts about 17 d and the amplexing period may be as long as 7 d (authors' personal observations), all experiments were started ~5 d after females molted, and were run until all females had either molted, died, or separated from their mates. Experimental replicates were run in 4-liter tanks. Pairs were inspected every 4 h around the clock (using a small hand-held lantern at night) to estimate guarding time. As pairs formed, they were removed and placed in individual 100 ml jars, and time until the female molted was recorded. To maintain the characteristic population structure of each particular test, each pair that was removed was always replaced by another male and female not in amplexus that had been held under similar conditions. Paired and unpaired individuals were blotted dry and weighed to the nearest 0.01 mg after each experiment.

Experiments

Two experiments tested the effect of the relative weight structure of the local male population on mean guarding time. The first experiment tested the effect of male wet weights on guarding time; weight within treatments were similar. Two treatments were conducted: in the first treatment all males were small (4.0 to 18.0 mg wet wt), and in the second treatment all males were large (20.0 to 34.0 mg wet wt). Wet weight of females was similar in both treatments (3.0 to 4.0 mg). The null hypothesis of no difference in mean guarding time due to male weight was tested using Welch's approximate t -test (t_c) (Davenport and Webster 1975) to account for unequal variances (Zar 1984). The second experiment tested for the effect of variance in the male wet weight-frequency distribution on precopulatory time. Two treatments were performed: in Treatment 1 the male wet weight range was greater (2.0 to 50.0 mg) than in Treatment 2 (8.0 to 22.0 mg). Eight males were used in each trial; there were four trials per treatment. After a 2 d period of acclimation for the males, eight females (which had molted within 5 d) were haphazardly collected from the laboratory population and added to the tanks. A t_c -test was used to test the null hypothesis of no difference in the mean guarding time due to variability in male weight-frequency distribution. A t_c -test was also used to test the null hypotheses of no difference in the mean male weight between treatments and in the mean weight of the males that entered amplexus in relation to the mean recorded for each treatment.

Four different sex ratios (male:female, 2:1, 1:1, 1:2, and 1:3) tested the effect of the operational sex ratio (OSR) on mean guarding time. Total density was 80 individuals per tank (± 4) and the variance of the male (range: 18.0 to 20.0 mg) and female (range: 3.0 to 4.0 mg) wet weight-frequency distribution was low. Four trials were run per treatment. An ANOVA was used to test the null hypothesis of no differences in mean guarding time (hours) among different operational sex ratios.

An experiment was conducted to assess the effect of the local population density on guarding time. Four male densities (treatments) were used (2, 6, 12 and 24 males per 4-liter tank); four trials were conducted per treatment. The variances in male wet weights among and within treatments were similar. After 2 d acclimation, females near the middle of their intermolt period were added to attain a sex ratio of 1:1. The tanks were monitored every 4 h for 7 d. An ANOVA was used to test the null hypothesis of no difference in mean guarding time (hours) among densities.

Results

In *Eogammarus oclairi*, mean guarding time (\pm SD) did not differ between small ($x = 55 \pm 20$ h, $n = 32$) and large ($x = 82 \pm 32$ h, $n = 32$; $t_c = 1.33$, $df = 60$, $p > 0.05$) male treatments, even though the mean weight of the males differed (small-male treatment: $x = 11.0 \pm 4.5$ mg, large-male treatment: $x = 27.0 \pm 4.6$ mg; $t_c = 14.5$, $df = 61$, $p < 0.05$).

The mean time (\pm SD) males spent in amplexus was greater in the high-variance treatment ($x = 54 \pm 21.3$ h, $n = 8$) than in the low-variance treatment ($x = 37 \pm 12.7$ h, $n = 12$, $t_c = 2.64$, $df = 11$, $p < 0.05$); the mean weight of males used did not differ between treatments (high-variance treatment: $x = 15.7 \pm 11.5$ mg, range = 2.0 to 30.0 mg, low-variance treatment: $x = 12.9 \pm 6.4$, range = 8.0 to 22.0 mg, $t_c = 1.2$, $df = 50$, $p > 0.05$). Males that entered amplexus in the high-variance treatment were larger than amplexing males in the low-variance treatment ($t_c = 2.41$, $df = 9$,

$p < 0.05$), and were larger than the mean of the population ($x = 30.46 \pm 18.11$ mg, range = 5.2 to 57.3 mg, $t_c = 2.21$, $df = 15$, $p < 0.05$). There was no difference between the mean weight of males that entered amplexus ($x = 14.4 \pm 6.06$ mg, range = 4.6 to 24.45 mg) and the mean population weight in the low-variance treatment ($t_c = 0.7$, $df = 17$, $p > 0.05$).

The mean guarding time (\pm SD) was affected by the OSR (2:1, $x = 88 \pm 32$ h; 1:1, $x = 86 \pm 20$ h; 1:2, $x = 52 \pm 22$ h; 1:3, $x = 43 \pm 24$ h; ANOVA, $F = 7.49$, $df = 3$, 54, $p < 0.001$). Mean guarding time for the 2:1 (male:female) OSR ranged from 72 to 168 h and was not different from that when the sex ratio was 1:1 (Tukey a-posteriori test, $p > 0.05$), but was significantly longer than the guarding time in the other two treatments (Tukey a-posteriori test, 1:2, $p < 0.05$, 1:3, $p < 0.05$).

The mean precopulatory guarding time (\pm SD) increased with density (2 males: $x = 19 \pm 11$ h; 6 males: $x = 39 \pm 9$; 12 males: $x = 42 \pm 10$ h; 24 males: $x = 50 \pm 12$ h; ANOVA, $F = 5.81$, $df = 3$, 20, $p < 0.05$). The variation in guarding time ranged from a few hours at the lowest density treatment to 3 d at the highest density treatment. Mean guarding time was not significantly different among the 6, 12 and 24 amphipod density treatments (Tukey a-posteriori test: $p > 0.05$), but was significantly lower (19 h) at the lowest density treatment (2 males per tank; Tukey a-posteriori test, $p < 0.05$).

Discussion

Precopulatory mate-guarding may be a time-investment strategy that enables males to optimize their reproductive success when mating is limited to brief periods of time during the female's reproductive period (Parker 1974; Ridley 1983). Female amphipods are receptive for only a short time after molting (Birkhead and Clarkson 1980; Borowsky 1985, 1988; Dunham et al. 1989; Dunham and Hurshman 1991; Iribarne 1994). Our results indicate that in *Eogammarus oclairi* several parameters associated with population structure affect the male investment in mate-guarding. The male reproductive response is dependent on male-male interactions. Males respond to the relative size of males, the operational sex ratio, and the density of males in experimental populations. Males guard longer when females are a limiting resource or when the rate of encounter with other males is higher.

Larger males guarded females for a longer time than smaller males. This result differs from Ward's (1983) observations on *Gammarus pulex*. It is unclear why large males spend more time guarding females, since they are able to outcompete smaller males in mixed-size populations. A possible explanation is the relative energy investment. The energy spent per unit of guarding

time relative to the female's size and fecundity may be smaller for larger males, and thus larger males may be able to carry females for more time. Larger males have a lower probability of losing a female when paired compared to smaller males, and so they are able to spend more time and energy without any risk of losing their investment to another male (Iribarne 1994). This advantage probably allows larger males to guard females for a longer time than smaller males (Ward 1983).

An alternative explanation for longer guarding time (i.e., greater investment) by larger males is that time spent in amplexus may be less costly than the energy required to attack and displace other males. Displacement success for *Eogammarus oclairi* under laboratory conditions is higher (30%) than for other amphipod species (e.g. 15% *Gammarus pulex*, Birkhead and Clarkson 1980). However, if displacement is an important mechanism in obtaining females under natural conditions, it may occur only when the encounter rate with amplexing pairs is high and only in certain habitats (Ward and Porter 1993; Ward 1993; but see Dick and Elwood 1993). Since larger males appear to have a lower cost of entering amplexus due to loading constraint (Adams and Greenwood 1983), and a lower chance of being displaced (Elwood et al. 1987), it should be advantageous for larger males to enter into amplexus sooner than small males (Elwood et al. 1987). Although our laboratory experiments with *E. oclairi* achieved similar results, field studies display the opposite pattern, with larger males spending less time in amplexus (Iribarne 1994). At this point we are unable to explain these differences in behavior. Further experiments manipulating size structure and density of the population may help to clarify this discrepancy.

Similar to *Assellus aquaticus* and *A. meridianus* (Manning 1980), as the ratio of males to females increased, amplexus was initiated earlier, even though early pairing may provide lower reproductive gain rates in terms of fertilized eggs per unit of time. Parker (1974) suggested that males will pair when the gain due to guarding is greater than the gain from further searching. When the proportion of males in the population is high, most females close to molt will be in amplexus. Thus, high intra-male competition will be associated with an earlier male response to cues shown by the female. By minimizing guarding time when the proportion of females is high, males minimize energy investment (Adams and Greenwood 1983; Robinson and Doyle 1985) and predation risk (Ward 1986; but see Verrell 1985 for opposite view), and also maximize the number of copulations during the mating season.

The density of potential mates can affect the type of mating system (Parker 1970; Wells 1977; Thornhill 1984; McLain and Boromisa 1987). Searching for mates can affect the OSR. Hubbell and Johnson (1987) and Crowley et al. (1991) used computer simulations to investigate the effects of density and male quality in male choosiness regarding female size. Both studies

found that choosiness increased when survival probabilities and mate encounter-rate (as density) were high. Crowley et al. showed that, in general, increased mate density resulted in more discriminatory mating and greater assortativeness. When encounters between potential mates were infrequent, random mating by both sexes was the common outcome. However, these models did not consider changes in the duration of precopulatory amplexus due to other changes in population structure. Our experiments show that there is an increase in precopulatory guarding time due to density when other population parameters are held constant (females were of similar weight range in each treatment, and thus we can rule out difference in choosiness due to female weight and its correlation with fecundity). One possible explanation is that at higher densities the number of encounters between males increase and intensify the competitive environment, a response similar to that triggered by a higher proportion of males to females. If this is true, then the male:male rather than the male:female encounter rate will trigger male mate-guarding. Another possible explanation is that female density affects the time of onset by increasing the stimulus to initiate amplexus due to the greater concentration of pheromone. Our experimental design does not enable us to discern between these alternative hypotheses.

In conclusion, several features of *Eogammarus oclairi* populations affected the time a male invested in guarding a female, and the condition which determined whether a given female was acceptable or not varied depending on population structure. The weight structure and the OSR of the population, and thus the number and size of potential competitors, were the most important factors. While other factors affected the guarding time in the order of hours, changes in the weight structure or operational sex ratio modified the time investment in the order of days. Males showed a very flexible mating behavior that should have served to maximize their reproductive success under a variety of population conditions.

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